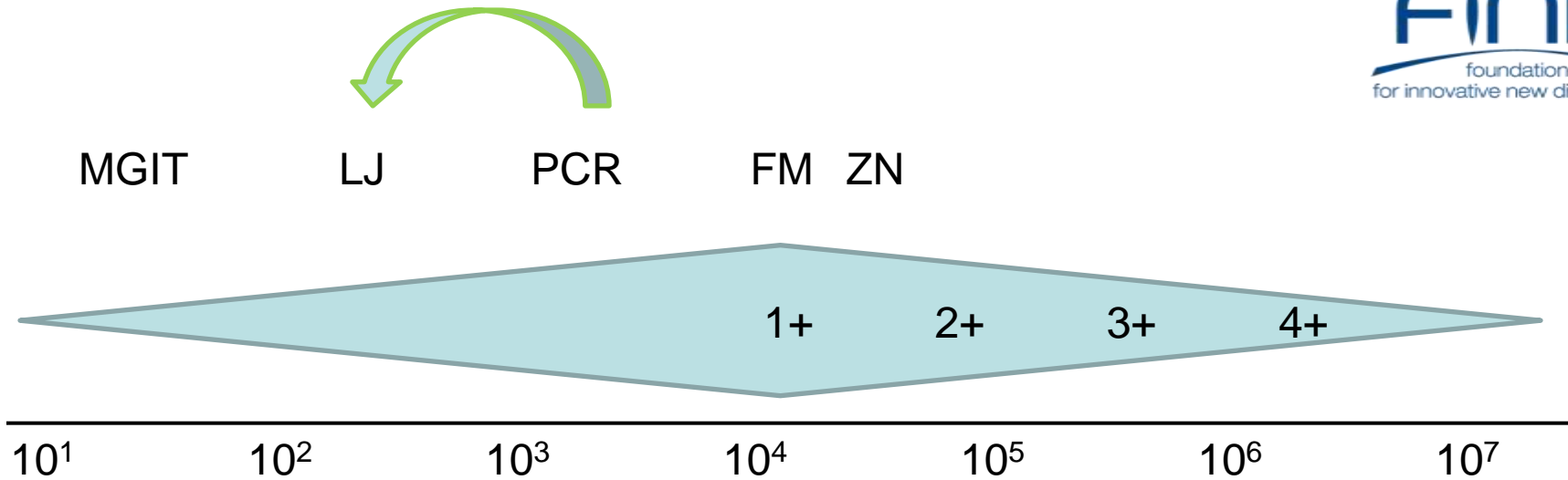


# Xpert MTB/RIF

Automated molecular detection of TB and MDR  
screening in peripheral laboratories

Mark Perkins, MD  
CSO FIND

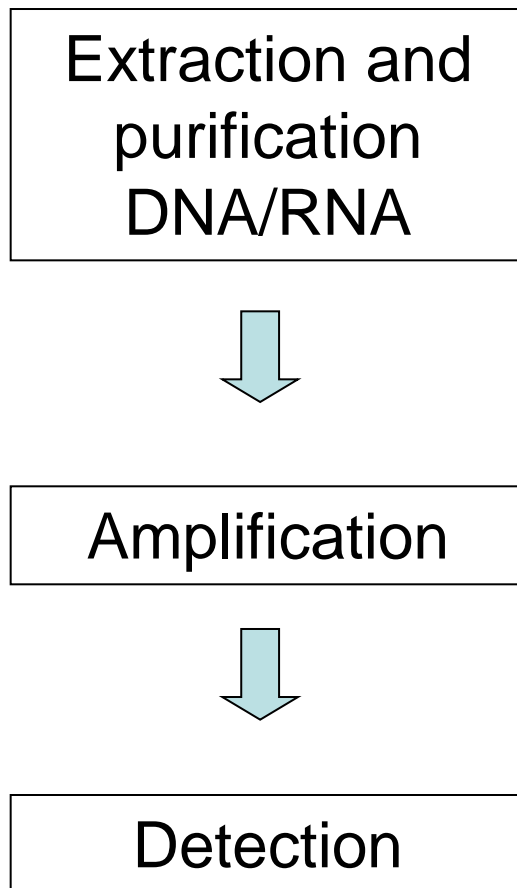


**Table 1: Pooled values (95% CI) of sensitivity and specificity of five commercial NAATs for pulmonary TB in 60 published studies (Greco, Girardi et al. 2006)**

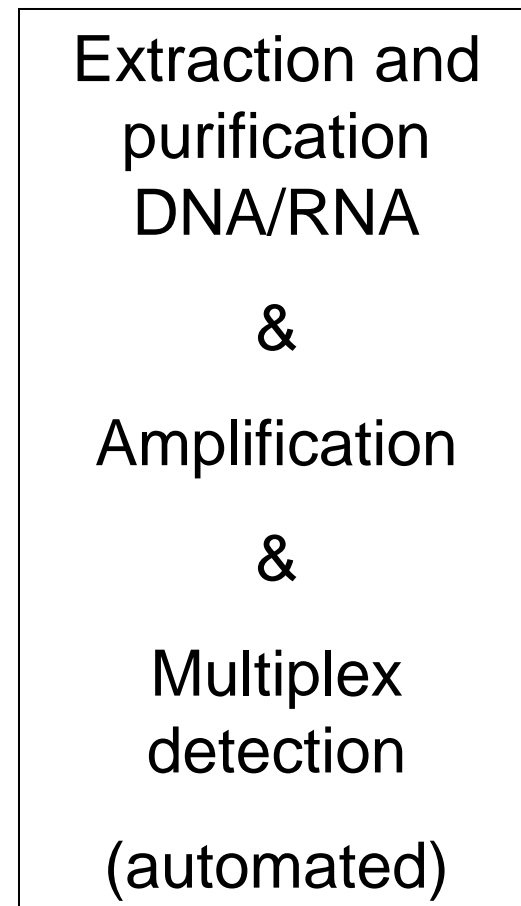
Test	AFB+		AFB-	
	Sensitivity	Specificity	Sensitivity	Specificity
Amplicor (PCR)	96 (94-97)	83 (80-86)	61 (57-65)	97 (96.8-97.4)
Cobas Amplicor (PCR)	96 (95-97)	74 (68-8)	64 (59-69)	99 (99.2-99.4)
BDP (SDA)	98 (96-99)	89 (84-93)	71 (66-76)	97 (96.4-97.4)
E-MTD (TMA)	97 (95-98)	96 (93-97)	76 (70-80)	97 (96.6-97.4)
LCx (LCR)	96 (94-98)	71 (64-78)	57 (50-64)	98 (97.8-98.5)

PCR: polymerase chain reaction; SDA: strand displacement amplification; TM: transcription mediated amplification; LCR: ligase chain reaction.

## Conventional NAAT



## Cepheid





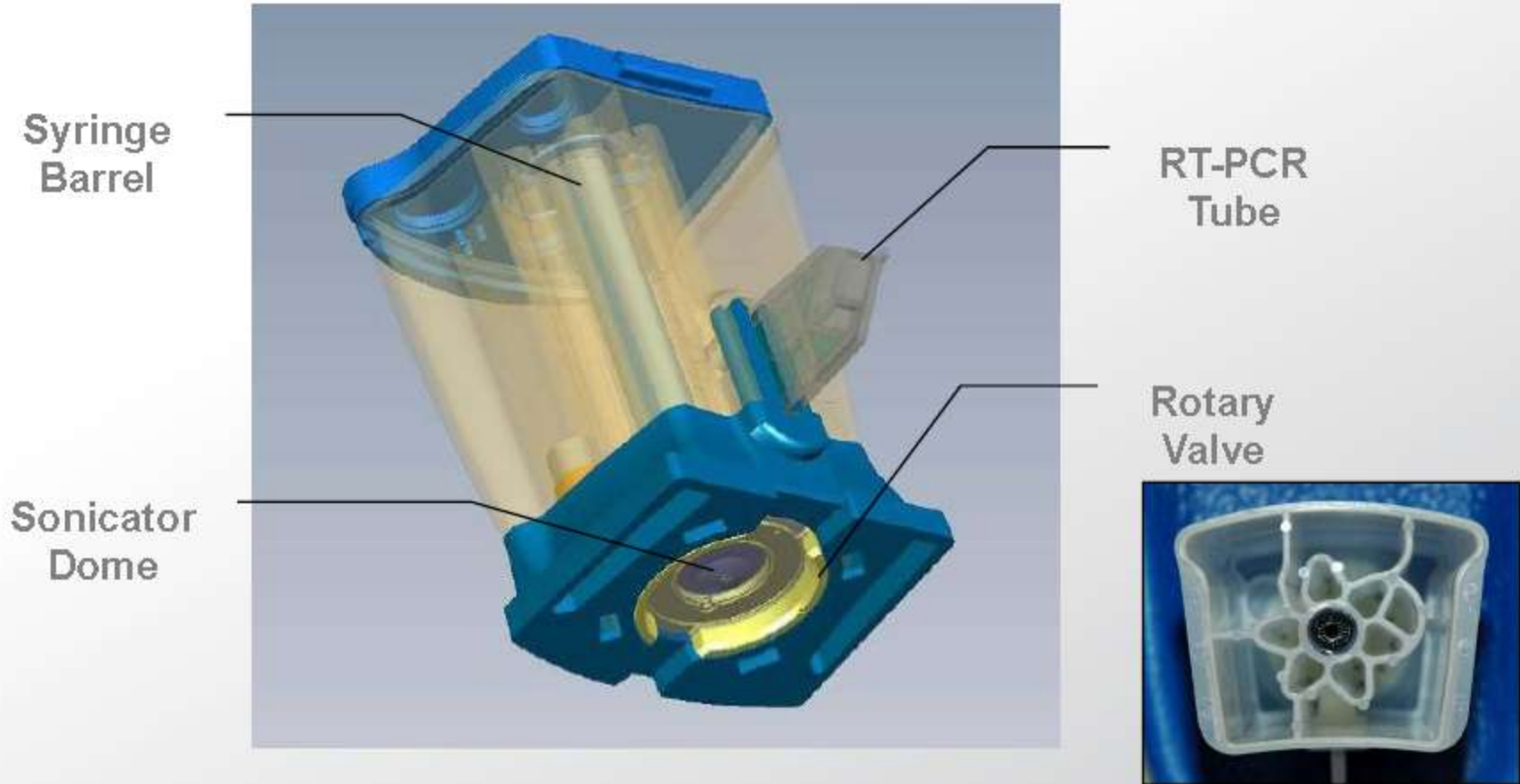
GeneXpert

Xpert  
MTB/RIF



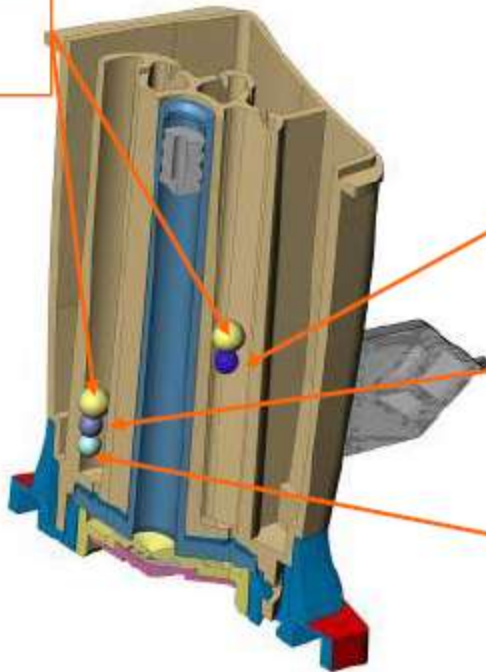
5	20	80	Samples per shift	500-1000
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# Cartridge Design and Operating Principle



# Bead Format Reagents

Retaining balls



Sample Processing Control bead:  
Bacillus globigii spores; excipients

Enzyme reagent bead:  
Taq polymerase; dNTPs; Buffers; Mg<sup>2+</sup>

Target-specific reagent bead:  
primers; rpoB specific probes; controls

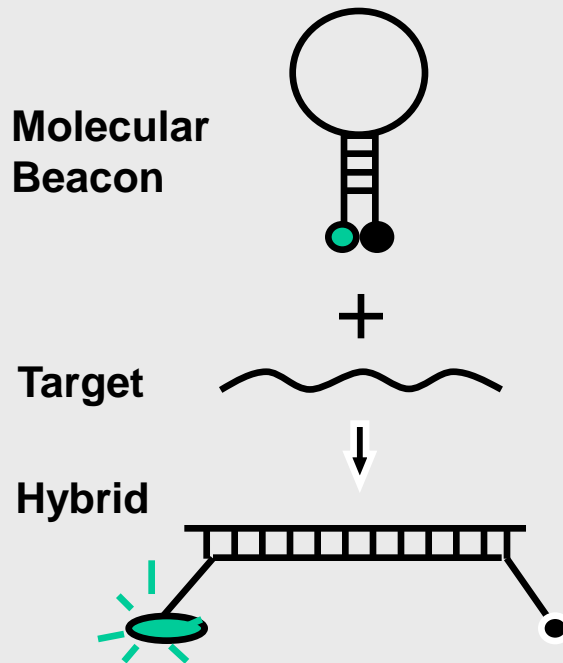
## Xpert MTB Cartridge Controls

- Probe Check Control
  - Is performed prior to PCR.
  - Is performed to ensure:
    - Rehydration of beads
    - Flow in cartridge works
    - That probe and dye are stable
- Sample processing control/extraction control
  - Ensure that the sample was correctly lysed and extracted.
  - Runs through the complete process
  - Is freeze-dried beads with *Bacillus globigii* spores . The spores are difficult to break open and to process.
  - Detects PCR inhibitors associated with the sample

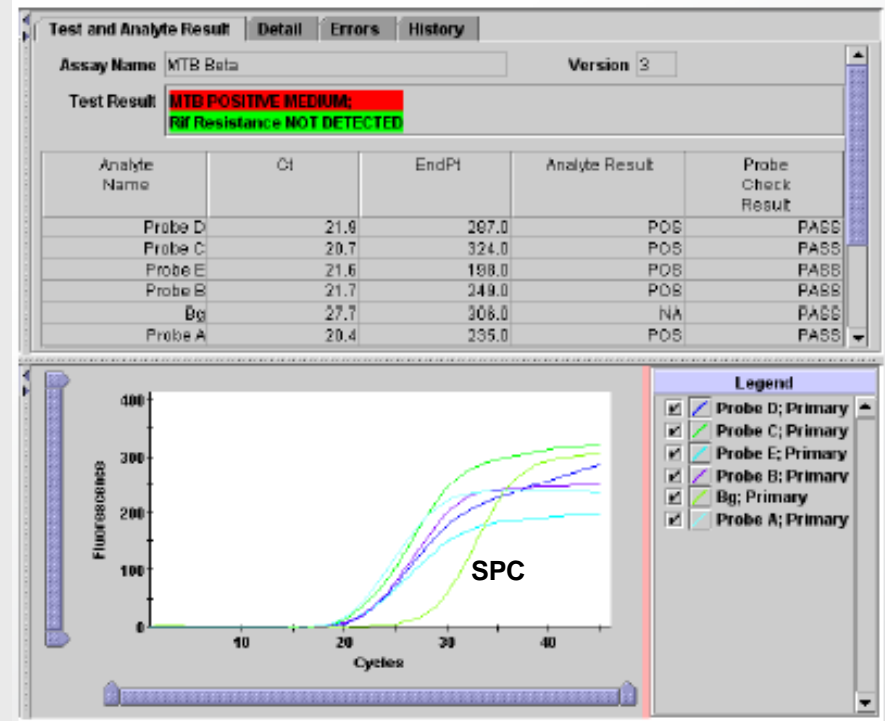
# Xpert MTB/Rif molecular beacon assay



The PCR target is the 81 bp region of the *rpoB* gene: 5 probes bind to wildtype, but not mutant target



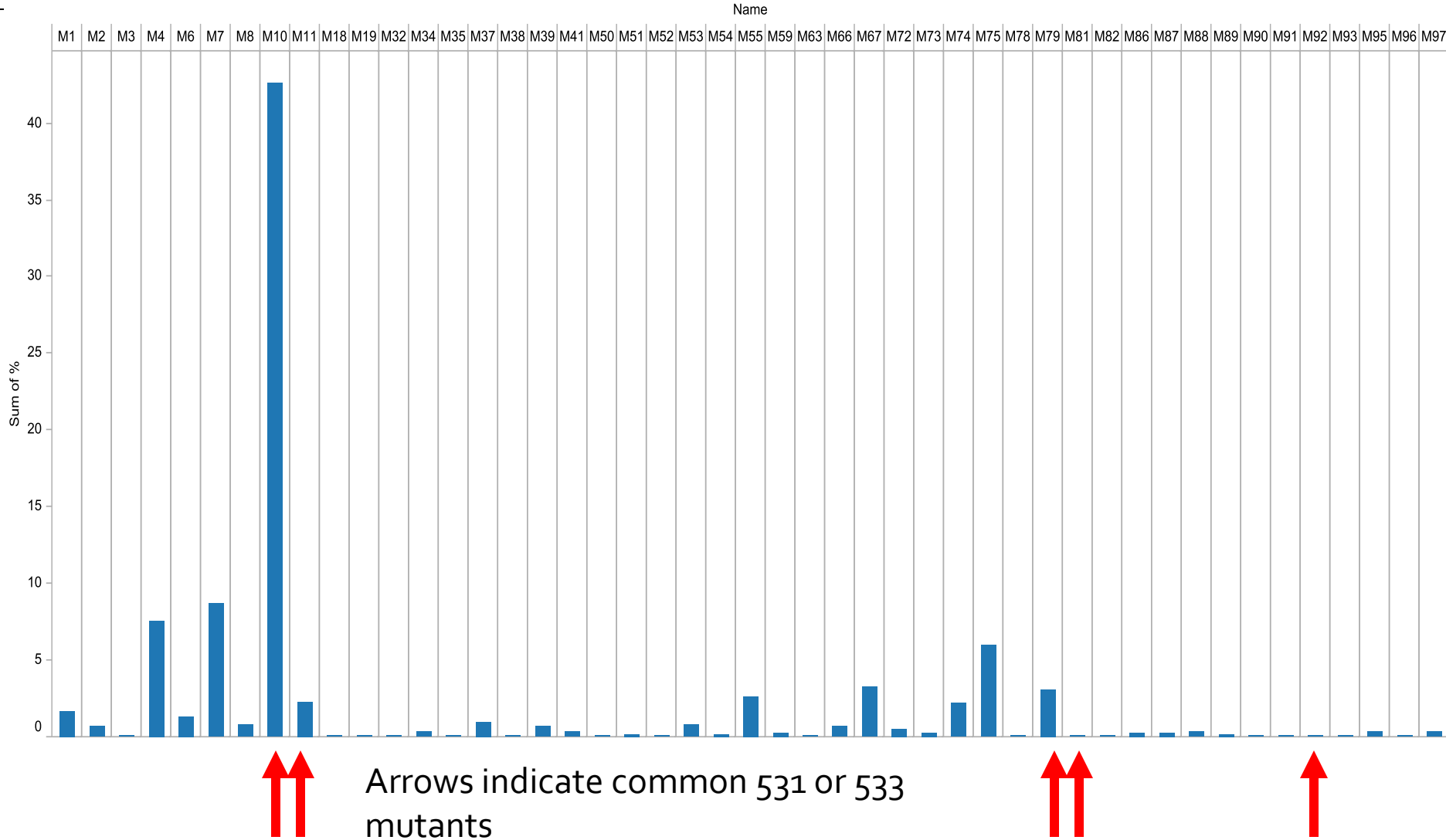
Each probe is labeled with a different fluorescent dye, permitting simultaneous detection



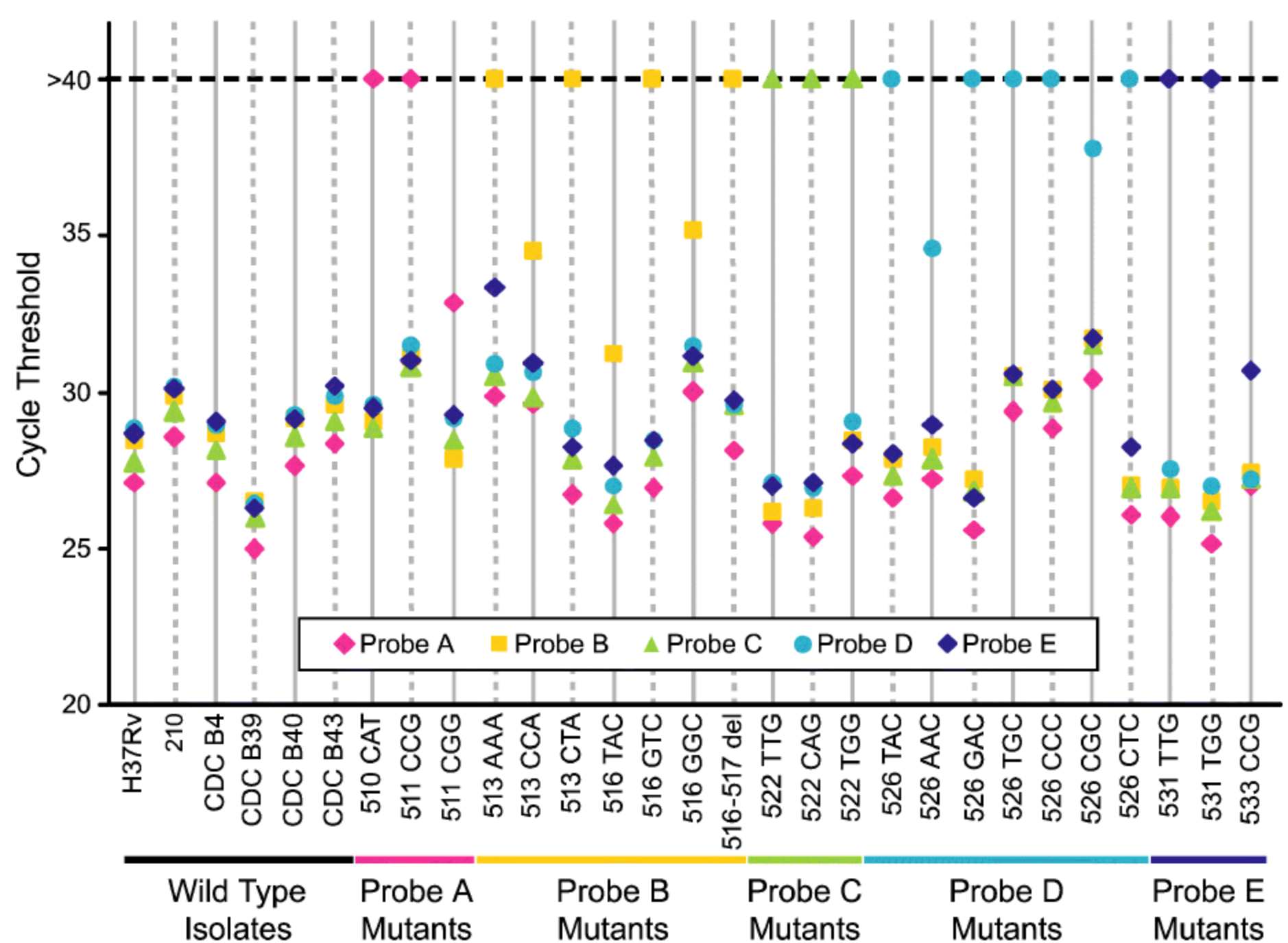
Example of Rif-Sensitive Profile – 5 probes & SPC show fluorescence



# Mutation Frequency



Frequency of mutation based on review of 4000+ strains



# Ubiquity Study



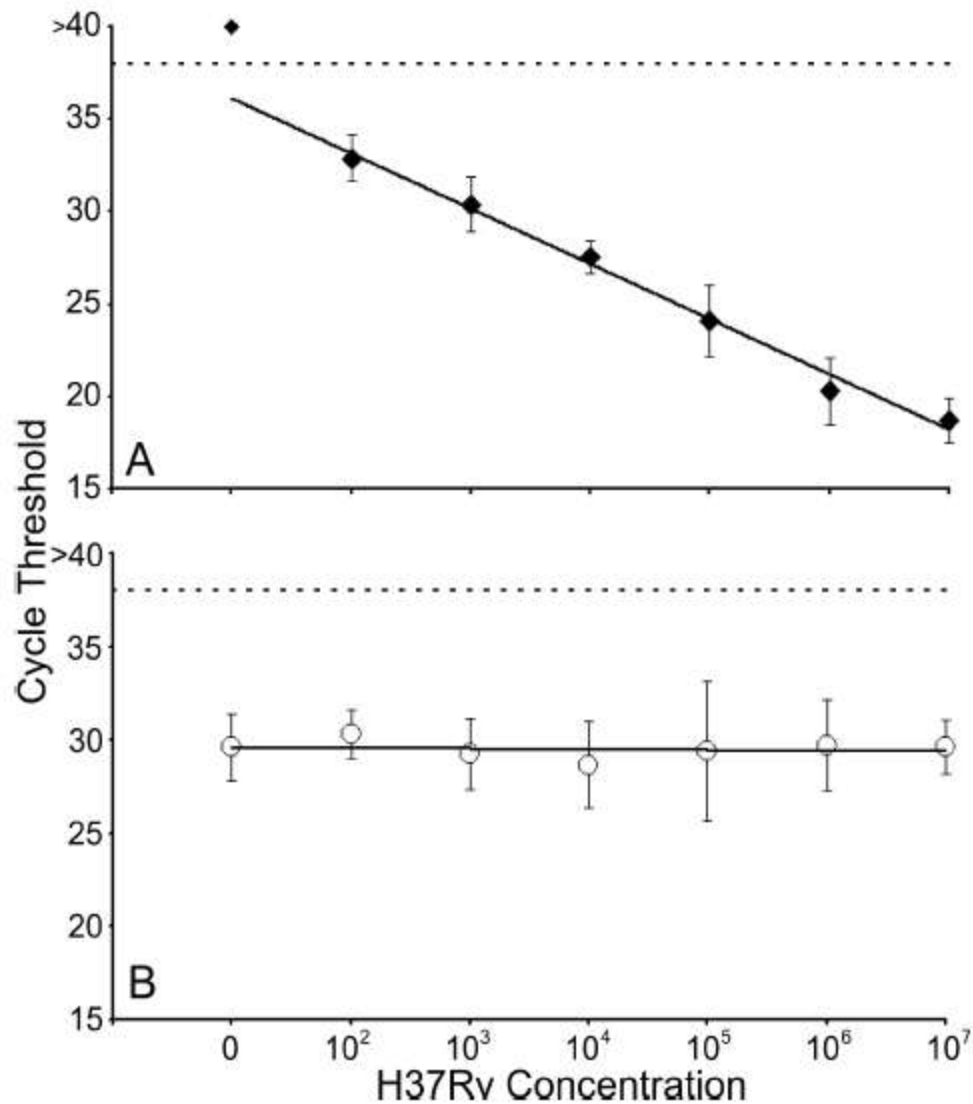
Origin	SCG								
	I	II	IIIa	IIIb	IIIc	IV	V	VIa	VIb
Unavailable	4	1			1		1		
Azerbaijan		1					1		
Bangladesh	2	3	4		1	1	1		
Belgium								1	
Burundi							2		
Camerun							1		
China		1							
Colombia						1			
Congo-Kinshasa							1	1	1
Georgia		1						1	
Germany							1	1	
Guinea				1					
Guinea Eq.							1		
India		1							
Kazakstan		1						2	
Korea		5	1	1					
Mexico					1	1			2
Morocco				1			1		
Nepal	1	1							
Nigeria							1		
Pakistan			1						
Peru				4	2	1	1		
Phillipines	1								
Portugal							1		
Rep. Domin.							1		
Rwanda								1	1
South Africa							2		1
Spain							1	2	
Tibet	1								
Ukraine								1	
USA						1			

- Tested dna from 80 strains from 30+ countries and 7 major clades.

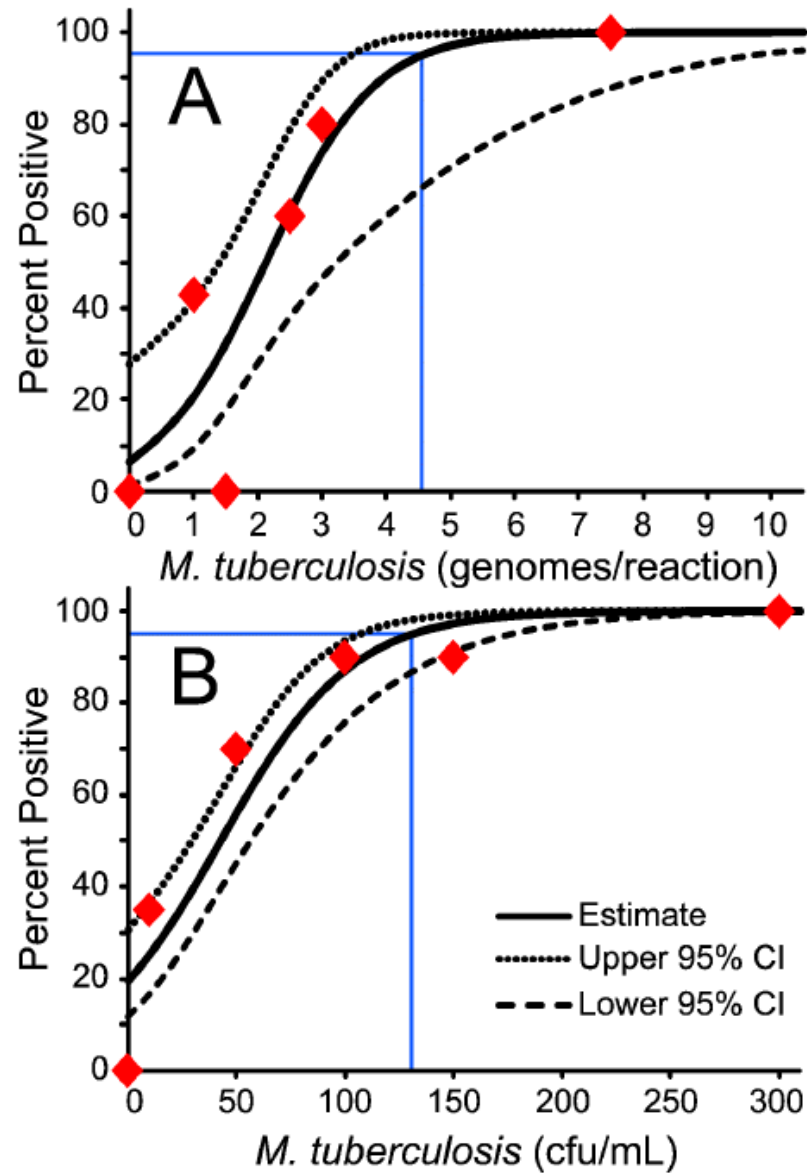
- Included 37 rifampin resistant strains comprising 13 unique mutations.

Codon	Mutation	Mut. Name	Probe
511	ctg-ccg	M1	A
516	gac-gtc	M4	B
516	gac-tac	M55	B
526	cac-tac	M7	D
526	cac-cgc	M67	D
526	cac-agc	M70	D
526	cac-gac	M75	D
531	tcg-ttg	M10	E
531	tcg-tgg	M79	E
533	ctg-ccg	M11	E
Double	multiple	M79+un (512)	E, AB
Double	multiple	M55+un (515)	B, B
Double	multiple	M67+un (509)	A, D

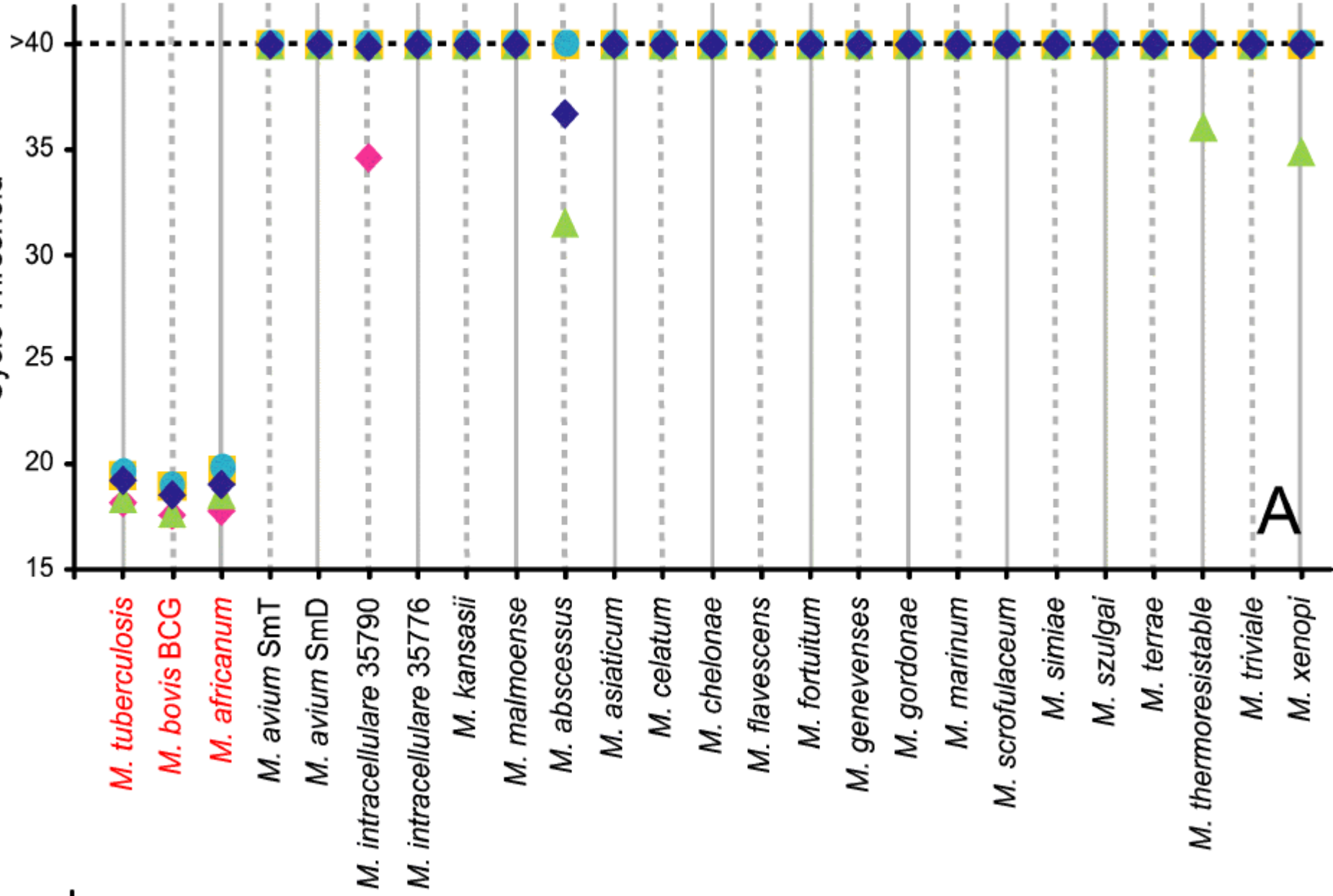
# Linearity of dynamic range



# Limits of detection

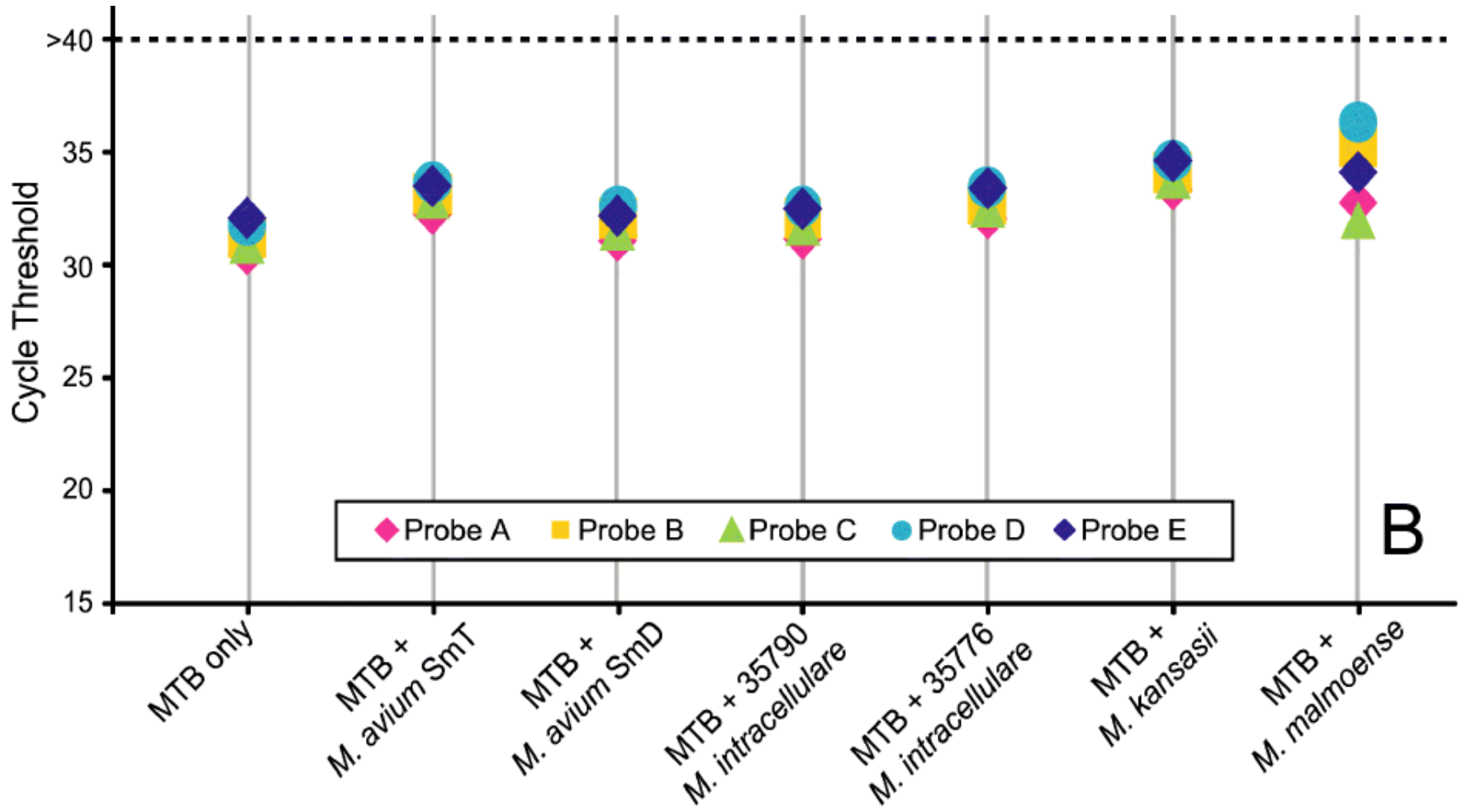


Cycle Threshold



A

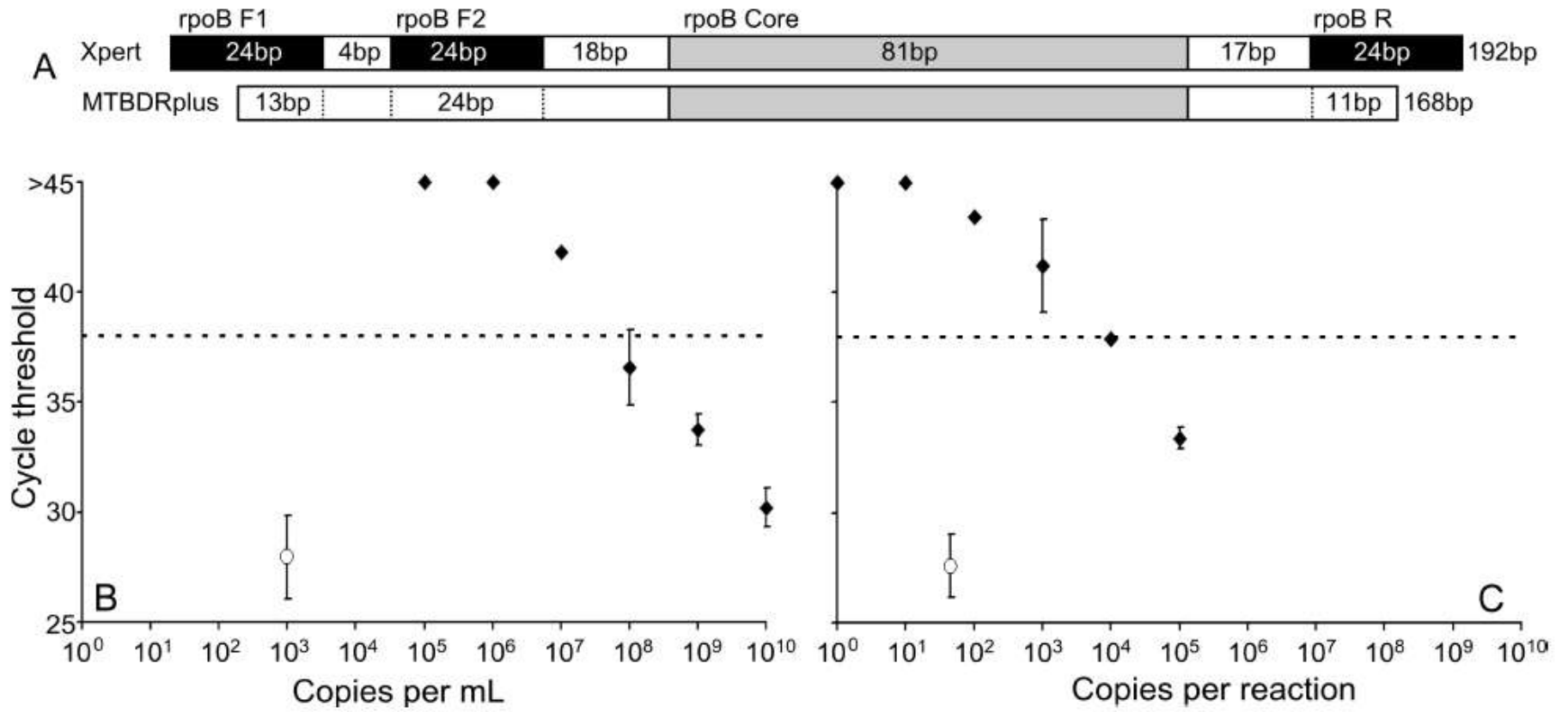
Interference: Sputum containing both 200 cfu/mL of *M. tuberculosis* and  $10^6$  cfu/mL of an NTM



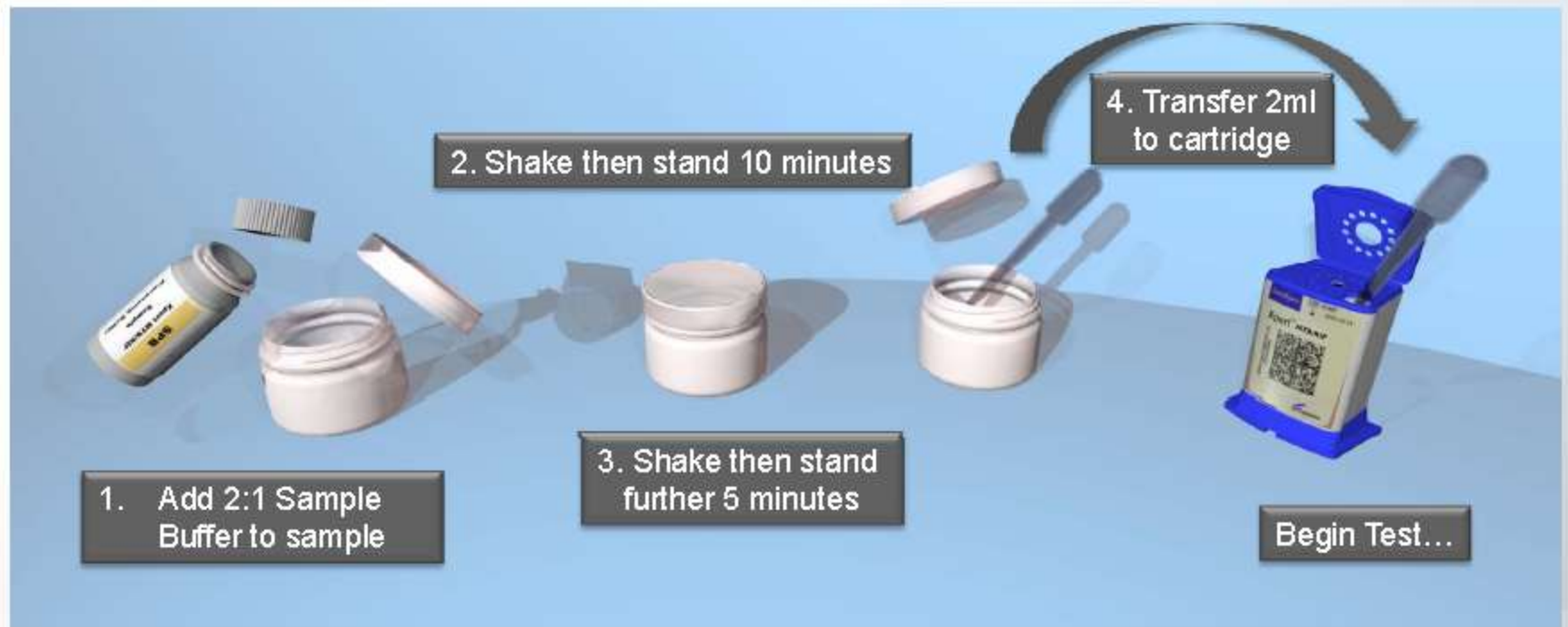
**B**





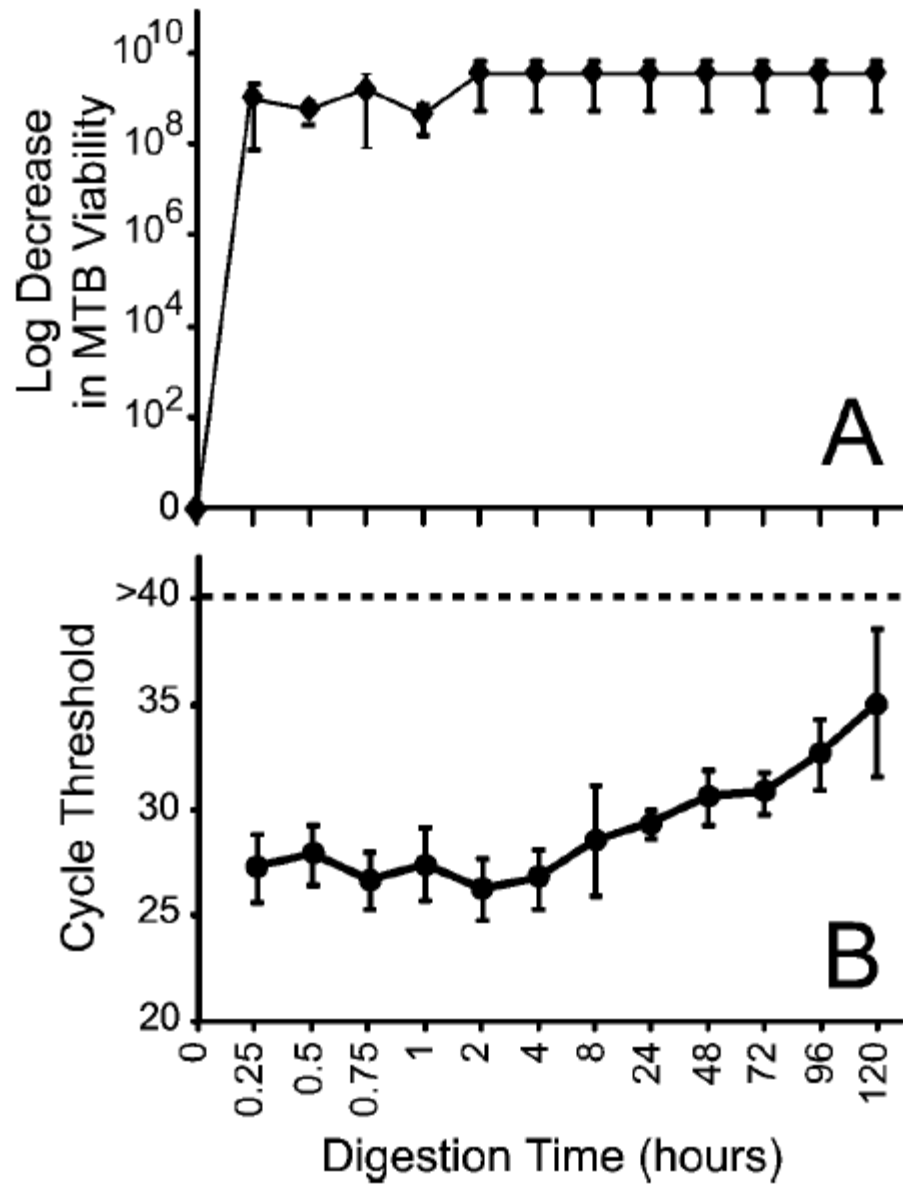


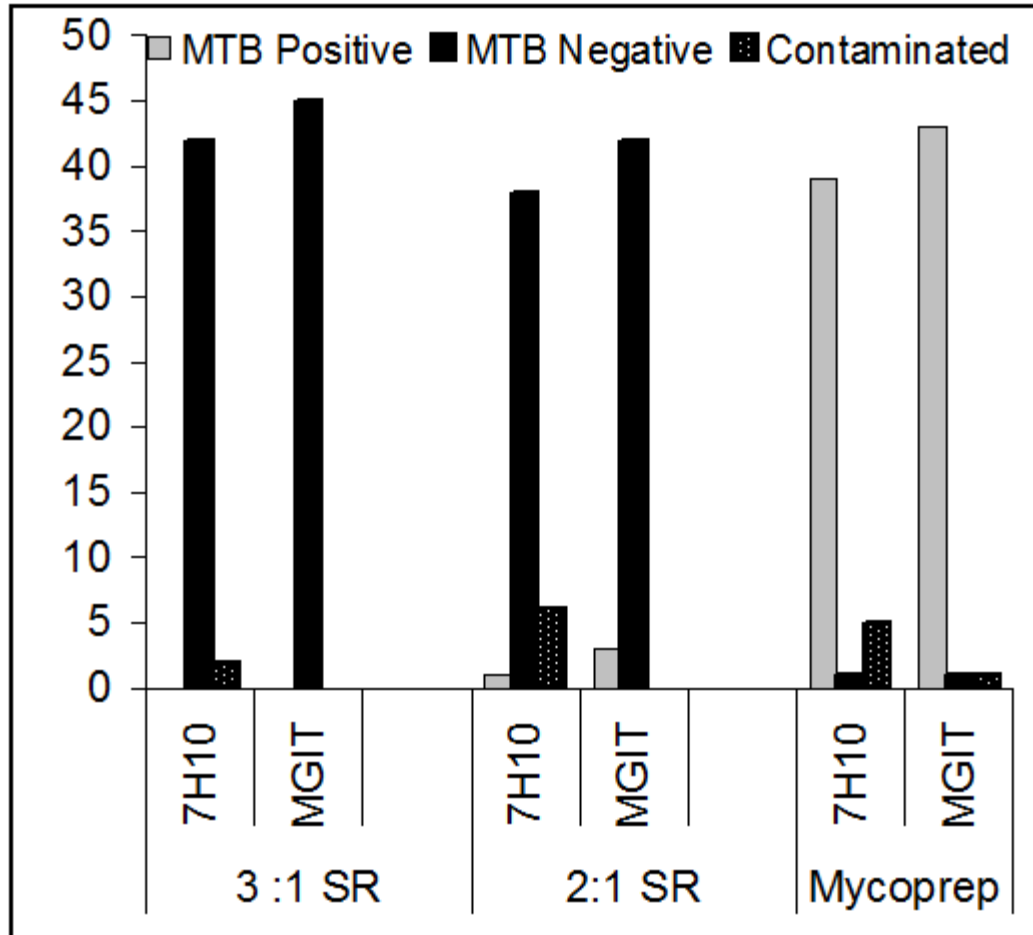
## Simple Sample Processing – Direct Sputum



# Inactivation procedure

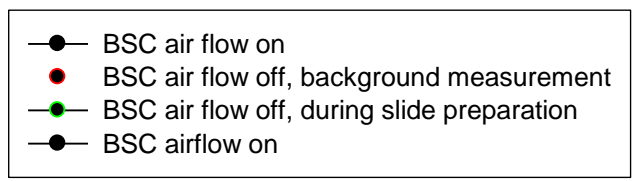
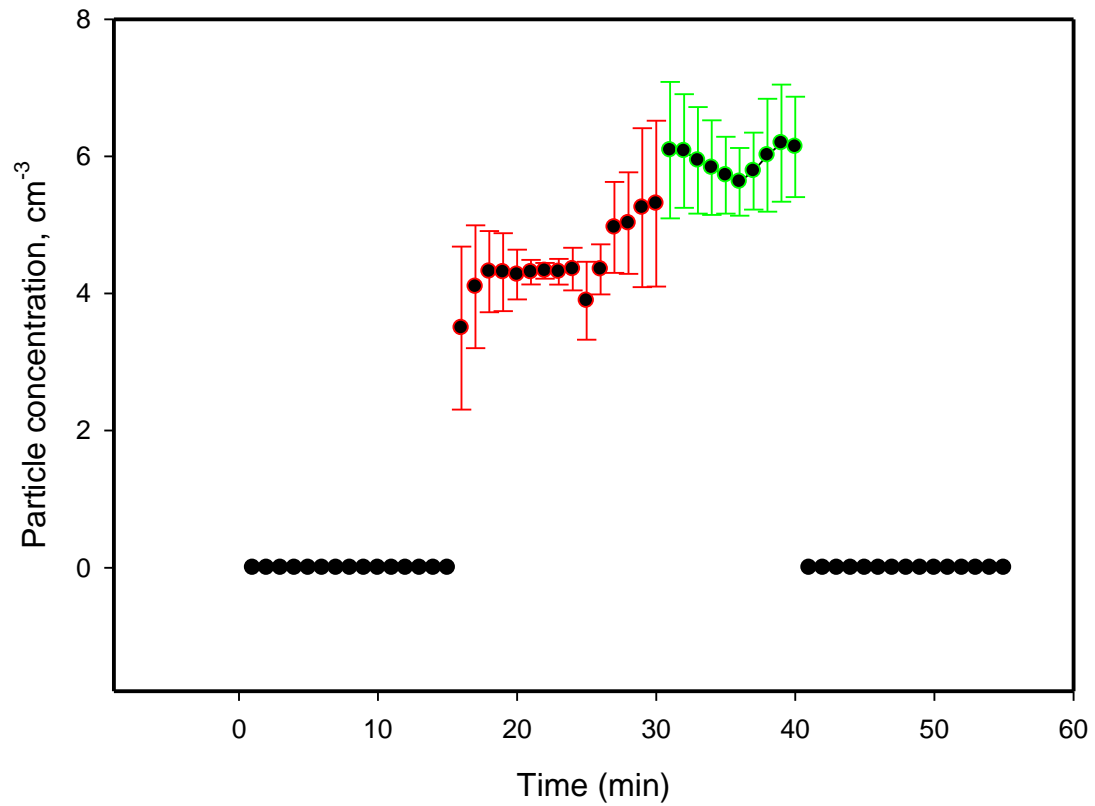
	Starting cfu/mL	Diluent	Average cfu/ plate for each replicate	Average cfu/plate	Average cfu/mL	Average log Reduction	Percent Reduction
Study 1	$3.5 \times 10^7$ BCG	7H9 media			<10	$>3.5 \times 10^6$	>99.9
Study 2	$3.5 \times 10^7$ BCG	sputum			1.5	$2.3 \times 10^7$	>99.9
Study 3	$3.3 \times 10^7$ H37Rv	sputum	12, 13, 21	15.3	153	$2.15 \times 10^5$	99.9
Study 4	$3.63 \times 10^8$ H37Rv	sputum	4, 6, 2	4	40	$9.1 \times 10^6$	>99.9
Study 5	$4.0 \times 10^8$ H37Rv	sputum	2, 2, 3	2.33	23.3	$1.7 \times 10^7$	>99.9
					<b>Average log kill</b>	<b><math>1.06 \times 10^7</math></b>	



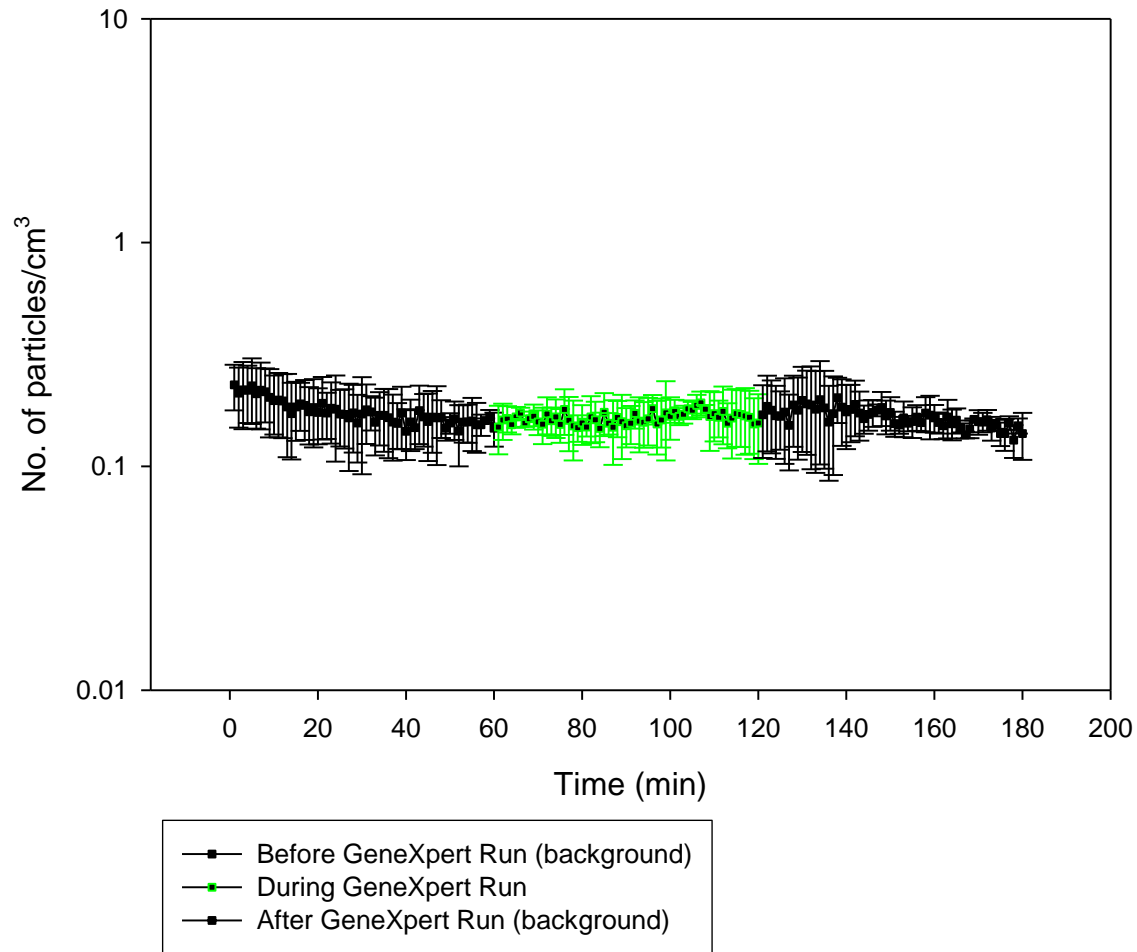


**Figure 12: Inactivation effectiveness of SR on smear-positive sputum**

### During conventional slide preparation



Concentration of airborne particles when GeneXpert is in operation  
(Sample - *Mycobacterium bovis* BCG + Sputum+SR)



Error bars indicates SD of three repeats

Biosampler  
Liquid media



Anderson Impactor  
Solid media





# Aerosol Sampling: verifying the system



0.8 mL BCG in 7H9 media aerosolized over 10 min in a closed chamber

Mean cfu/m<sup>3</sup> air detected for 3 experiments

<u>Concentration BCG/mL.</u>	<u>Anderson impactor</u>	<u>BioSampler</u>
10 <sup>4</sup>	482	10,416
10 <sup>3</sup>	169	2,976
10 <sup>2</sup>	91	578
10	28	0

# Aerosol Viability During Manual Steps

Mean cfu/m<sup>3</sup> air detected over 3 experiments

5 X 10<sup>8</sup> cfu BCG spiked into sputum.

Anderson impactor

BioSampler

SR added and sample **immediately** pipetted in and out of three Xpert TB cartridge over 15 min time period (equivalent to loading >30 cartridges)

6

67

SR added **15 min wait** then sample pipetted in and out of three Xpert TB cartridge over 15 min time period (equivalent to loading >30 cartridges)

0

0

Sputum smeared/layered on 10 microscope slides over 10 min period.

16

324

# Aerosol Viability During GX Automated Assay Steps

Total cfu detected over all three runs

Sample type placed into Xpert TB cartridge (3 runs with 3 cartridge per condition)

Anderson impactor

BioSampler

5 X 10<sup>8</sup> cfu BCG spiked into water

0

0

5 X 10<sup>8</sup> cfu BCG spiked into sputum then treated with SR in standard protocol

0

0

5 X 10<sup>8</sup> cfu M. smegmatis spiked into sputum then treated with SR in standard protocol

0

0

